Lethal and Sublethal Effects of Potash Brine on Different Stages of the Lobster, *Homarus americanus*

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St. Andrews, N. B., E0G 2X0

January 1985

Canadian Technical Report of Fisheries and Aquatic Sciences No. 1344

85-2391/

Canadian Technical Report of Fisheries and Aquatic Sciences

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LETHAL AND SUBLETHAL EFFECTS OF POTASH BRINE ON DIFFERENT STAGES OF THE LOBSTER, *Homarus americanus*

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This is the one hundred and seventy-nineth Technical Report from the Biological Station, St. Andrews, N. B.

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Cat. No. Fs 97-6/1344E ISSN 0706-6457

Correct citation for this publication:

Charmantier, G., M. Charmantier-Daures, and W. W. Young-Lai. 1985. Lethal and sublethal effects of potash brine on different stages of the lobster, Homarus americanus. Can. Tech. Rep. Fish. Aquat. Sci. 1344: iii + 13 p.

ABSTRACT

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Denison Mine, Sussex, New Brunswick, proposes to discharge a brine, prepared by diluting potash ore in well water, into the Bay of Fundy. To set criteria for the dilution of this brine in seawater (SW), we studied the biological repercussions of different ore concentrations on various developmental stages of the American lobster, Homarus americanus. Acute toxicity bioassays enabled us to calculate the 96 h LC50s, which at 20°C ranged from 1 to 2 g ore/L SW in larval stages I, II, and III, and 2.25 to 3 g/L in stage IV and juveniles. At 11°C, LC50s are roughly similar, except in stage IV, 1.5 to 2.25 g/L. Sublethal bioassays showed that the development from hatch to stage IV was only possible if the concentration was under 1.5 g ore/L SW.

Comparative experiments using "Instant Ocean" salts showed that the cause of death is not related to an increase in osmolality of the medium. Other experiments using additions of NACl or KCl showed that the principal toxic agent in the brine was the K^+. K^+ titrations in the blood of lobsters which were exposed to different concentrations of ore for different durations indicate that cause of death could be the toxic effect of increased blood K^+ concentrations.

RÉSUMÉ

Charmantier, G., M. Charmantier-Daures, and W. W. Young-Lai. 1985. Lethal and sublethal effects of potash brine on different stages of the lobster, Homarus americanus. Can. Tech. Rep. Fish. Aquat. Sci. 1344: iii + 13 p.

La Mine Denison, Sussex, Nouveau-Brunswick, projettent de rejeter une saumûre, préparée par dilution de minerai de potasse dans une eau de puits, dans la Baie de Fundy. Afin d'établir des critères de dilution de cette saumûre dans l'eau de mer (EM), nous avons étudié les répercussions biologiques de différentes concentrations de minerai sur divers stades de développement du Homard Américain, Homarus americanus. L'analyse chimique du minerai a montré une forte proportion de KCl qui représente 47% de son poids (K^+: 24.8%). Des essais biologiques de toxicité aiguë nous ont permis de calculer les CL50-96 h (concentrations léthales pour 50% des animaux): à 20°C, elles sont de 1 à 2 g minerai/L EM pour les stades larvaires I, II et III, et de 2.25 à 3 g/L pour le stade IV et les juvéniles. A 11°C, les CL50 sont à peu près les mêmes, sauf en stade IV, où elles sont de 1.5 à 2.25 g/L. Des essais biologiques sub-léthaux ont montré que le développement, de la naissance au stade IV, était possible seulement si la concentration était inférieure à 1.5 g minerai/L EM.

Des expériences comparatives avec des sels "Instant Ocean" ont montré que la cause de la mort n'était pas liée à une augmentation de l'osmolalité du milieu. D'autres expériences, avec ajout de NaCl ou KCl, ont montré que le principal agent toxique de la saumûre était le K^+. Des dosages de K^+ dans l'hémolymphe de homards soumis à différentes concentrations de minerais durant des temps variables montrent que la cause de la mort pourrait être l'effet toxique de concentrations élevées de K^+ dans l'hémolymphe.

INTRODUCTION

The construction of potash mines in New Brunswick is accompanied by problems related to brine disposal. Denison Mine, Sussex, New Brunswick, Canada, proposes to use a saturated solution of the ore in well water for the transportation of the mined potash in the plant and discharge the excess brine into the Bay of Fundy. The effluent will therefore contain a high proportion of potassium salts.

The impact of this brine solution on the marine biota, in general, and on the lobster, in particular, poses many unanswered questions. To set limits for the tolerable concentrations of the brine effluent, we studied its effects on larval, postlarval and juvenile stages of the lobster.

In natural systems, hypo-salinity is more prevalent then hyper-salinity; hence the information available on the effect of hyper-salinity is less comprehensive (Emery and Stevenson 1957; Daniel and Boyden 1975; Mantel and Farmer 1983). In Homarus, while the effect of low salinity on larval development and behavior is relatively well documented (Gompel and Legendre 1927; Templeman 1936; Scarratt and Raine 1967; Sastry and Vargo 1977), very few data exist on the susceptibility of lobsters to high salinity seawater (Templeman 1936; Sastry and Vargo 1977). Studies on the effect of salt brine discharges on crustaceans are also scarce. The most comprehensive study was undertaken by Howe et al. (1982) on penaeid shrimps.

The main objective of the present study was to determine the maximum tolerable concentration of the brine effluent in the vicinity of the outfall from Denison Mine. To achieve this objective we studied the survival of larval and postlarval Homarus americanus in high salinity seawater and in different concentrations of potash brine in seawater. We also attempted to explain some of the physiological effects of the brine on the lobster by studying the osmotic regulation and the regulation of K^+ ion in the hemolymph, using juvenile lobsters, the size of which was more amenable to experiments and titrations.

MATERIALS AND METHODS

TEST ANIMALS

Larvae were obtained from July to September from lobsters held in the lobster culture facility at the Biological Station, St. Andrews, N. B., Canada. After hatching, larvae were transferred to 40 L larval plankton kreisels (Hughes et al. 1974) and supplied with flow-through seawater of 30 °/oo salinity. Temperature was maintained at 20°C and photoperiod was natural. Larvae were fed with frozen brine shrimp (Artemia). Most tests were conducted on stages I to V in molt stage C. These animals were selected in the middle of each larval or postlarval stage, the duration of which was known from daily observations and data on larval development (Templeman 1936). For tests requiring postmolt lobsters, the individuals were collected after hatching (I A) or molting (II A, III A, IV A); premolt larvae (stage D) were collected when approximately 50% had molted to the following stage. The number of lobsters per test concentration was usually 20 or 10, but sometimes 5. Juvenile lobsters were between 20-55 mm carapace length.

BIOASSAYS

Ninety-six hour, acute, static bioassays were conducted with lobsters held individually in compartmented 5-L plastic, covered, aerated containers filled with 3.5 L of test medium. Lobsters were not fed during the bioassay. Tests were conducted at 20 ± 1°C and at 11 ± 1°C in Environment Controlled Temperature rooms (Coldstream). These temperatures were selected because at 20°C larval development is optimal, well documented (Templeman 1936) and predictable, and 11°C represents the average, ambient summer temperature of the seawater along the coast of the Bay of Fundy. Lobsters were transferred directly from seawater to each test solution. Containers were inspected at logarithmic intervals from 0.5 to 96 h (Sprague 1969).

Sublethal bioassays were also conducted. Within 24 h after hatching stage I A lobster were transferred to the test medium. Lobsters in higher salinities were acclimated for 1 h per test increment, starting from the ambient seawater and using 20 individually held lobsters per test concentration. Lobsters were fed daily with frozen brine shrimp after uneaten food was removed. The experiments terminated when all the lobsters molted to stage IV. Test solutions were changed every 4 d.

During exposure to the different test media the criteria of death were total lack of movement, no beating of the scaphognathite and no response after repeated touches with a probe. Once death was verified the lobsters were removed.

The hatch from one female was used for each experimental series. Most of these bioassays were duplicated, especially at the concentrations near the lethal threshold.

PREPARATION OF TEST MEDIUM

High salinity test waters were prepared by adding dry "Instant Ocean Synthetic Sea Salts" (Aquarium Systems, Inc.) to ambient seawater. In acute bioassays the concentration of test solutions ranged from seawater (approximately 900 m Osm/kg) to 1700 m Osm/kg, with increments of 100 m Osm/kg. Long-term bioassays were run in ambient seawater and media of 1000, 1100, and 1200 m Osm/kg osmotic pressure.

Test concentrations of ore were prepared using brine corresponding to a nearly saturated ore solution (270 g/L) while actual saturation concentration is approximately 300.5 g/L. The 270 g/L solution was prepared with dried, crushed ore, diluted in Denison Mine well water from the Denison Mine site. The saturated solution was filtered before addition to seawater to prepare a dose series. In acute bioassays the concentrations of ore in seawater (g of ore/L of seawater) were as follows: 0, 0.5, 1.0, 1.25, 1.50, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.5, 4.0, 5.0, 7.0, 10.0, 15.0. Concentrations of 0, 0.5, 1.0 and 1.5 g/L were used for long-term bioassays.

Other control experiments were conducted by using potash ore diluted directly in seawater and by using KCl and NACl solutions in well water.

The osmotic pressure of the test media was measured with an Advanced Instruments Osmometer. Corresponding salinities were measured by using a refractometer (Sopelem), an hydrometer (Ertco, S.G. - 1.000-1.500), a portable salinity meter (Yellow Springs Instruments, Model 33), and a salinometer (Autosal, Model 8400).

TITRATIONS

Hemolymph was removed from juvenile lobsters with a 1 mL syringe inserted into the sinus at the base of the fifth pereiopod. Hemolymph osmotic pressure was measured on an Advanced Instruments Osmometer. K^+ concentrations were measured by atomic absorption spectrophotometry (Perkins-Elmer 303).

ANALYSIS OF DATA

Median times to 50% mortality (LT50) were estimated by graphic approximation to probit analysis (Litchfield and Wilcoxon 1949). Median lethal concentrations (LC50) and confidence intervals were estimated by standard programs of probit analysis (Finney 1952; Davies 1971). Lethality curves were estimated by using the Letcur program (Zitko 1982).

RESULTS

The composition of the potash ore, "Instant Ocean", seawater and well water is summarized in Table 1. The percentage of K^+ in the potash ore was 24.8 % compared to 1.1 % in "Instant Ocean" and 1.3 % in seawater. In Table 2, some of the brine concentrations tested with corresponding osmotic pressures and salinities measured by refraction, specific gravity and conductivity are given. Conductivity measured with the YSI salinity meter seemed to give the most accurate value of salinity in parts per thousand. From these and other measurements, it appears that the addition of 1 g ore/L SW increased the salinity by about 0.82. In other measurements of osmotic pressure and salinity, a value of 3.3 °/oo is equivalent to 100 m Osm/kg, which will be used for converting osmotic pressure to parts per thousand.

EFFECTS OF INCREASED SALINITY ("INSTANT OCEAN")

Acute bioassays

At 20°C the limit of survival for lobster stages I to IV A was 1200-1300 m Osm/kg (40.8-44.2 °/oo). Stage IV C were capable of surviving in higher salinities, 1300-1400 m Osm/kg (44.2-47.6 °/oo) (Table 3). At 11°C the limits of survival for all stages, I to IV, were 1200-1300 m Osm/kg (40.8-44.2 °/oo) (Table 3). When the susceptibility of animals of different molt stages was compared in 1300 and 1400 m Osm/kg, stages I A, IV C, IV D, V A and V C all showed LT50s exceeding 96 h in 1300 m Osm/kg (Fig. 1). At 1400 m Osm/kg only stages IV C, IV D and V C experienced LT50 over 96 h (Fig. 1).

Sublethal bioassays

The 1200 m Osm/kg (39.6 °/oo) group all died in 24 d. The 1100 Osm/kg (36.3 °/oo) group had 78% mortality. The control seawater group was 60% and the lowest mortality was 40% in the 1000 m Osm/kg (33 °/oo) group (Fig. 2). Except for the 1200 m Osm/kg concentration all mortalities occurred during or shortly after molting. A salinity of 1200 m Osm/kg (39.6 °/oo) was the upper limit for molting of larval lobsters (Fig. 3). High salinities also affected larval development (Fig. 4). In 1200 m Osm/kg larval development rate was retarded; 15 d were required to complete the molt from stage I to stage II. Meanwhile the other groups, 1100, 1000 and SW, had already completed the second stage and were well into the third stage. The fastest development rate occurred at 1000 m Osm/kg, 24 d; seawater was 26 d and 1100 m Osm/kg was 28 d from hatch to stage IV.

Overall, the 1000 m Osm/kg group showed the best performance in terms of survival and development rate. Development rates for all larval stages, I, II and III, were 6, 9 and 10 d, respectively, which were consistently lower than all the other salinities.

EFFECT OF ORE BRINE

Acute bioassays

Shortly after introduction into brine concentrations in excess of 3 g/L, lobsters changed from their natural blue-green color to red-green. Contact with the brine solution was typified by an avoidance reaction in which larvae and postlarvae swam rapidly for a few seconds. This was followed by a quiescent period, lying on their sides with chelipeds and pereiopods extended and pleopods beating rapidly. In brine concentrations exceeding 10 g/L lobsters were paralysed in less than a minute and died in less than 30 min. Juvenile lobsters did not display the same color changes as larval or postlarval lobsters. Juveniles that were severely stressed would lie on their backs with tails extended and pereiopods moving slowly back and forth until they succumbed.

The mortality curve for 20°C lobsters (Fig. 5) shows no difference from stages I to III. However, there was an obvious difference between the larval stages (I to III) and the postlarval stages (IV and juveniles). At 11°C there is no difference between the larval and postlarval stages, indicating that susceptibility to potash brine increased at lower temperatures. The 96-h median LT50 with confidence intervals are presented in Tables 4a, b, c.

The median lethal concentrations (LC50, 96 h) ranged from 1 to 2 g/L for larval stages I to III. In stage IV and juvenile lobsters the median lethal concentrations were higher - 2.25 to 3.0 g/L at 20°C (Table 5). At 11°C the LC50s for stages I to III stages I to III ranged from 1.25 to 2 g/L with a slight increase to 1.5 to 2.25 for stage IV.

Stages III and IV tested in KCl gave the same results as the experiments in brine at 20°C (Table 5). Stage IV lobsters exposed to 7 and 10 g NaCl/L did not experience any mortalities after 8 d (Table

6). When the ore was dissolved directly in seawater there was no difference in mortality from that of the saturated solution prepared in well water (Table 6).

Sublethal bioassays

In 1.5 g/L, 100% mortality occurred after 13 d, 90% in the 1.0 g/L, 75% in 0.5 g/L and 60% in the control seawater (Fig. 6). Mortalities were over 50% in all the groups in spite of being reared individually.

At 1.5 g/L larval development was inhibited. After 11 d none of these larvae was successful in molting to stage II. In the other groups the rate of larval development to stage IV was fastest in the 1.0 g/L lobsters, 21 d, 23 d for the 0.5 g/L and 28 d for the control group (Fig. 7).

Short-term exposure of stage III lobsters to 3 g/L for 1 h and 4 g/L for 0.5 h did not affect survival and molting to stage IV.

OSMOREGULATION AND K^+ REGULATION

Adaptation time

After transfer of juvenile lobsters in stage C from seawater to 2 g ore/L SW (a concentration compatible with the survival of these animals), the K^+ content of hemolymph increases and stabilizes in 12 to 24 h (Fig. 8). A similar experiment with a lethal concentration of 5 g ore/L SW results in a continuous increase in the osmotic pressure of hemolymyh in 5 h up to a value slightly above the osmotic pressure of the medium (Fig. 9); the K^+ content of hemolymph increases up to 3 h after transfer, then stabilizes until the death of the animals (Fig. 10).

Osmotic and K^+ Regulation

Using a time of adaptation of 24 h in each medium, we studied the osmoregulation and K^+ regulation in seawater containing different concentrations of either "Instant Ocean" salts or ore. After addition of ore, the osmotic pressure of the medium is only slightly increased and the osmotic regulation of the lobsters is slightly hyper-osmotic as in lobsters maintained in "Instant Ocean" SW solutions (Fig. 11). In the same way, the type of hypo-ionic K^+ regulation in hemolymph seems not to be altered even in the very high K^+ concentrations obtained by addition of up to 2.5 g ore/L SW (Fig. 12).

DISCUSSION

In high salinity media, prepared by adding "Instant Ocean" sea salts to SW, the median lethal concentrations (LC50, 96 h) were 1200-1300 or 1400-1500 m Osm/kg (39.6-42.9 or 46.2-49.5 °/oo) depending on the molt stage and the developmental stage. When the different stages of development were compared, larval stages I to III were found to be less resistant to increases in salinity than postlarval stage IV and juveniles. This could be attributed to the greater ability to osmoregulate which appears after metamorphosis, i.e. from stage IV (Charmantier et al. 1984, in press). Development from hatch to stage IV is possible in media up to 1100 m Osm/kg (36.3 °/oo). Higher concentrations (1200, 1300 m Osm/kg; 39.6, 42.9 °/oo), although lower than the LC50, generally prevent molting to the next stage.

After adding brine prepared with potash ore from Denison Mine, LC50s ranged from 1 to 2 g ore/L SW in larval stages I, II, III, and 2.25 to 3 g/L in stage IV and juveniles (1.5 to 2.25 g/L in stage IV at 11°C). As was found previously after addition of "Instant Ocean" salts, stage IV postlarvae and juveniles were more resistant to the brine than larvae.

Since adding 3 g ore/L only increases the osmotic pressure of the medium approximately 50 m Osm/kg, the lethal effect of the brine is not caused by an increase in osmotic pressure or by heavy metal toxicity (Table 1). The toxic effect is caused by K^+. This was verified when control experiments using additions of NACl did not cause mortalities; in retrospect when KCl was added to SW the response of the animals was similar to the response in the brine media. Addition of 1 g ore/L SW adds about 5 m Eq K^+/L, i.e. increases the K^+ concentration in the medium about 65%. Our K^+ titrations, in juveniles, show that they are able to regulate the K^+ concentration in hemolymph in media containing up to 2.5 g ore/L SW which corresponds roughly to the LC50. Up to this concentration the patterns of osmotic and K^+ regulation did not seem to be altered. If exposed to more concentrated media the the animals die when their hemolymph K^+ concentration surpasses 20 m Eq K^+/L, i.e. about 170% above the average hemolymph concentrations for animals kept in SW. High blood concentrations of K^+ are known to be lethal by affecting muscular contraction, especially the cardiac muscle. It is very likely that the mechanism is similar during exposure to brine. Further intracellular K^+ titrations will verify this hypothesis.

From these results a few guidelines can be proposed for setting the maximum concentration of ore in SW that can be tolerated in the mixing zone from the Denison Mine outfall. The maximum ore concentration in SW should not surpass the LC50s found for larval stages I to III because these stages are the most sensitive to the brine. The LC50s ranged from 1 to 2 g/L, depending on the larval stage and the temperature (Fig. 5, Table 5). Development from hatch to stage IV can only be accomplished in concentrations below 1.5 g/L. However, the LC50s are higher if the exposure time decreases. The tolerance zone, shown on the left of the mortality curve (Fig. 5), represents the combinations of concentrations/times that are compatible with the survival of the animals. For example, we found that after exposure of stage III to 3 and 4 g/L for 1 and 0.5 h, respectively, these animals survived and molted successfully to the following stage. However, we do not know what the effects would be for repetitive exposures at high concentrations for short durations. This is an area which should be considered for future work.

Since we are dealing with such low concentrations of brine it is very critical that the release of the brine (saturated solution of ore at 300 g/L) should be carefully controlled at its source. Salinity measurements in the field should be done by using an accurate method, preferably a conductivity measurement (Table 2).

The likelihood that lobsters will encounter concentrations of brine equivalent to those used in these experiments will depend on the behavior of the different stages and the dispersion rates and distribution patterns of the discharge. Stages I to III larvae are planktonic stages capable of migrating vertically for several meters in the water column. Stage IV is a transition stage, between planktonic and benthic, and from late stage IV onwards lobsters become benthic. Depending on local hydrographic conditions, the dispersion pattern of the brine may be highly variable. If the brine is effectively diluted and dispersed it may affect both planktonic and benthic stages. If it has a tendency to settle on the bottom, due to high specific gravity, then it will affect only the benthic and transition stages. A knowledge of the hydrographic conditions around the discharge site would therefore be necessary for the application of these biological data.

ACKNOWLEDGMENTS

This study was carried out at the Biological Station, St. Andrews, N.B., Canada, with financial assistance from the National Research Council of Canada and the Ministère français des Relations extérieures. Special thanks go to Dr. D.J. Scarratt for initiating the project, for his liaison with Denison Mine officials and for his interest during the study, and to Dr. D.E. Aiken for providing the animals, use of his facilities and comments on the manuscript.

We thank Les Burridge for advice on bioassays, A. Sreedharan for statistical analysis, Vicki Jerome and Debbie Martin-Robichaud for chemical analysis of the potash ore, Dr. D.H. Loring for heavy metal analysis, Eugene Henderson for the loan of the osmometer, Frank Cunningham for preparation of the figures, Barbara Garnett for typing the manuscript.

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Table 1. Composition of well water, seawater, potash ore and "Instant Ocean" Salt.

Ion	Well water (g/L)	Seawater (g/L)	Potash ore (g/kg)	"Instant Ocean" Salt (g/kg)
Cl^-		17.0	483.3	553.6
Na^+		9.9	170.5	306.9
K^+	$2.12\ 10^{-3}$	0.375	248.1	11.1
Mg^{++}	$0.097\ 10^{-3}$	1.06	0.030	36.1
Ca^{++}	$0.25\ 10^{-3}$	0.35	0.628	11.1
Zn	$1.37\ 10^{-6}$		$\sim 3\ 10^{-3}$	
Cd	$0.95\ 10^{-6}$		$20\ 10^{-6}$	
Cu	$1.74\ 10^{-6}$		$\sim 1\ 10^{-3}$	
Fe	$12.96\ 10^{-6}$		0.5	
Pb	-		$\sim 1\ 10^{-3}$	

Table 2. Brine concentrations tested with corresponding osmotic pressure (m Osm/kg) and salinities (°/oo) estimated by four methods.

Ore concentration (g/L)	Osmolality (a) (m Osm/kg)	Salinity (°/oo) (b)	(c)	(d)	(e)
0	944	28.5	30.8	30.4	31.3
0.5	955	28.5	30.8	30.9	31.8
1.0	968	28.5	32.1	31.3	32.2
1.5	974	28.5	32.1	31.9	32.6
2.0	980	31.5	33.0	31.9	32.3
2.25	985	31.5	33.0	32.1	33.6
2.5	989	31.5	33.0	32.5	33.9
3.0	998	33.0	33.0	33.0	34.3
4.0	1015	33.0	33.0	33.8	35.2
7.0	1068	34.0	33.5	36.0	37.3

(a) Advanced Instruments Osmometer
(b) Sopelem refractometer
(c) Ertco hydrometer, S.G. 1.000-1.050
(d) Yellow Springs Instruments salinity meter (Model 33)
(e) Autosal salinometer (Model 8400)

Table 3. Median lethal concentrations (LC50, 96 h) for lobsters held in high (H) salinities at 20°C and 11°C. Concentrations are expressed in m Osm/kg. Stages not tested are indicated by N.T. Molt stages tested were postmolt (A), intermolt (C) and premolt (D).

Temp.	Salinity	Larval stages I A	I C	I D	II A	II C	II D	III A	III C	III D	IV A	IV C
20°C	H	←			1200-1300			→ ←	1300-1400	→ ←	1200-1300 →	1400-1500
11°C	H	1300-1400	1200-1300	N.T.	N.T.	1200-1300	N.T.	N.T.	1400-1500	N.T.	N.T.	1300-1400

Table 4a. Median lethal time (LT50, 96 h) for larval and juvenile lobsters at 20°C; 95% confidence intervals indicated.

	I C		II C		III C		IV C		Juv. (25-30 mm)	
Brine	LT50	Lower upper	LT50	Lower upper	LT50	Lower upper	LT50	Lower upper	LT50	Lower upper
0	-		-		-		-		-	
0.50	-		-		-		-		-	
1.00	-		-		-		-		-	
1.25	-		96	*	81.3	61.5 107.1	-		-	
1.50	86.5	46.1 162.3	21.2	7.6 59.4	81.2	65.3 101.2	-		-	
1.75	71.3	55.2 92.0	29.7	21.6 40.6	82.6	64.3 106.1	-		-	
2.00	13.0	3.7 13.9	9.5	6.9 13.2	18.4	12.9 26.3	-		-	
2.25	11.7	9.1 15.1	7.3	5.2 10.3	8.1	6.3 10.4	-		-	
2.50	10.1	7.2 14.0	5.7	3.9 8.3	7.2	5.4 9.6	23.4	17.5 31.2	-	
2.75	6.4	4.7 8.7	2.9	1.8 4.4	3.9	2.9 5.4	18.3	14.1 23.8	-	
3.00	2.5	*	1.7	1.1 2.7	3.7	2.7 5.1	5.9	4.5 7.8	11.0	*
3.50	2.2	1.6 3.1	0.8	*	1.5	1.1 2.2	2.8	1.6 4.8	-	
4.00	1.1	*	0.6	*	0.57	0.50 0.65	1.6	1.03 2.60	6.0	4.2 8.6
5.00	0.35	0.28 0.43	0.47	*	0.41	0.38 0.43	0.83	0.76 0.92	4.5	3.6 5.5
7.00	0.33	0.28 0.38	0.31	0.28 0.35	0.27	*	0.71	0.65 0.77	2.6	2.2 2.9
10.00	0.28	0.25 0.35	0.24	0.21 0.27	0.24	0.21 0.27	0.67	0.61 0.74	-	
15.00	0.16	0.12 0.24	0.18	0.16 0.21	0.11	0.06 0.20	0.52	0.50 0.54	-	

- LT50 > 96 h.
* Insufficient data to calculate C.I.

Table 4b. Median lethal time (LT50, 96 h) for larval and juvenile lobsters at 11°C; 95% confidence intervals indicated.

Brine conc. (g/L)	I C		II C		III C		IV C	
	LT50	Lower upper	LT50	Lower upper	LT50	Lower upper	LT50	Lower upper
0		-		-		-		-
0.50		-		-		-		-
1.00		-		-		-		-
1.25		-		-		-		-
1.50		-	94	*		-		-
1.75	99.0	70.5 139.2	28.2	28.1 28.3	26.2	13.4 51.3		-
2.00	48.4	41.8 55.9	31.3	25.6 39.2	18.0	*	15.3	1.3 183.2
2.25	43.8	36.6 52.4	16.1	10.7 24.0	11.5	6.8 19.3	19.0	9.5 37.8
2.50	13.2	8.9 19.5	8.0	5.3 11.9	6.5	3.3 13.0	11.0	7.3 16.4
2.75	6.2	3.8 10.1	2.1	1.4 3.2	2.2	1.7 2.9	8.0	4.6 13.8
3.00	1.6	0.9 2.9	1.4	1.1 1.7	1.6	*	3.1	2.2 4.5
3.50	1.0	*	1.3	*	1.4	*	1.3	0.8 1.9
4.00	0.8	*	0.80	*	1.3	*	0.43	0.32 0.58
5.00	0.47	0.43 0.52	0.53	*	0.54	0.48 0.61	0.26	0.23 0.30
7.00	0.43	*	0.34	0.31 0.39	0.39	0.36 0.42	0.25	0.24 0.29
10.00	0.36	*	0.25	0.20 0.31	0.35	0.32 0.38	0.21	0.18 0.24
15.00	0.22	0.17 0.29	0.19	0.14 0.25	0.30	0.27 0.35	0.13	0.12 0.14

- LT50 > 96 h.
* Insufficient data to calculate C.I.

Table 4c. Medial lethal time (LT50, 96 h) for lobsters in KCL; 95% confidence intervals indicated.

KCL conc. (g/L)	III C		IV C	
	LT50	Lower upper	LT50	Lower upper
0		-		-
0.50		-		-
1.00		-		-
1.25		-		-
1.50	52.2	33.0 82.3		-
1.75	51.1	36.9 70.9		-
2.00	18.0	*		-
2.25	14.0	*		-
2.50	5.9	4.4 7.9		-
2.75	5.6	4.0 7.9	14.2	*
3.00	2.9	2.2 3.9	5.8	*
4.00	0.54	0.48 0.61	1.8	1.39 2.33
7.00	0.27	*	0.70	0.69 0.76
10.00	0.18	0.14 0.22	0.65	0.59 0.71

- LT50 > 96 h
* Insufficient data to calculate C.I.

Table 5. Median lethal concentrations (LC50) in g/L for larval, postlarval and juvenile lobsters calculated by three methods.

Tests	Stages I	II	III	IV	Juveniles
			Potash ore (20°C)		
(1)	1.7	1.5	1.5	2.4	2.6
(2)	1.5 -1.7	1.1-1.4	1.1-1.4	2.4 -2.5	-
(3)	1.25-2.0	1.0-1.5	1.0-2.0	2.25-2.75	2.25-3.0
			Potash ore (11°C)		
(1)	1.8	1.7	1.8	2.1	
(2)	1.3 -1.5	-	1.2 -1.5	1.3 -1.6	
(3)	1.25-2.0	1.25-1.75	1.25-2.0	1.75-2.25	
			KCl (20°C)		
(1)			1.7	2.6	
(2)			1.2-1.8	-	
(3)			1.0-2.0	2.25-2.75	

(1) Lethality Curve Program (Zitko 1982).
(2) Probit Analysis (Finney 1952).
(3) Binomial Test (Horn 1956).

Table 6. Median lethal times for stage IV lobsters in NACl medium and potash ore diluted in seawater; no mortality = N.M., 95% confidence intervals indicated.

Concentration (g/L)	NACl	Ore diluted in SW 20°C		11°C	
0		N.M.		N.M.	
0.5		N.M.		N.M.	
1.0		N.M.		N.M.	
1.25		N.M.		N.M.	
1.5		N.M.		N.M.	
1.75		N.M.		N.M.	
2.0		N.M.		11.9	7.7 18.7
2.25		40.3	24.9 64.9	10.6	4.5 25.3
2.50		8.4	5.9 11.9	7.9	4.6 13.8
2.75		4.6	-	4.6	
3.0		4.4	-	5.5	
3.5		4.0	-	2.4	1.7 3.4
4.0		1.2	0.6 2.3	1.6	
7.0	N.M.	N.M.		N.M.	
10.0	N.M.	"		"	
	Binomial	1.75-2.5		1.5-2.0	

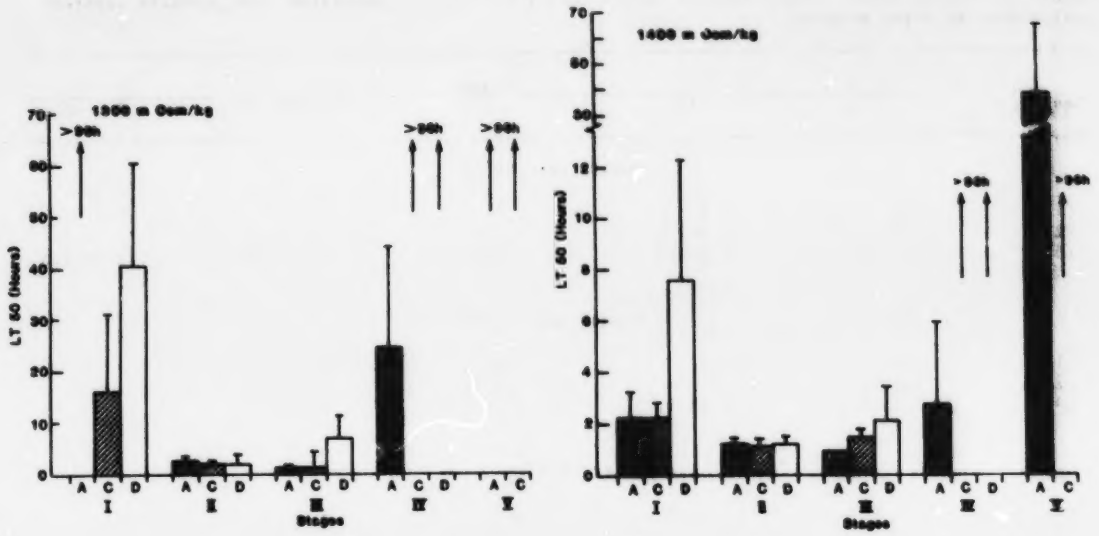

Fig. 1. Median lethal time (LT50, 96 h) for media concentrations 1300 and 1400 m Osm/kg at different times of the molt cycle - postmolt (A), intermolt (C) and premolt (D); 95% confidence interval indicated.

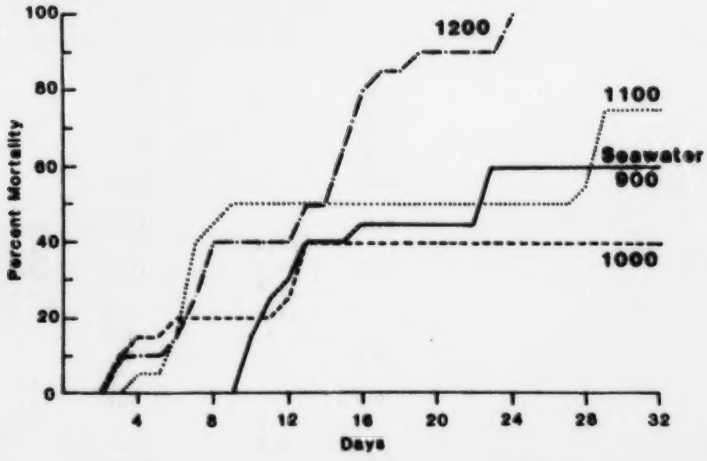

Fig. 2. Cumulative percent mortality of larvae and postlarvae reared from stage I in different salinities; concentrations in m Osm/kg.

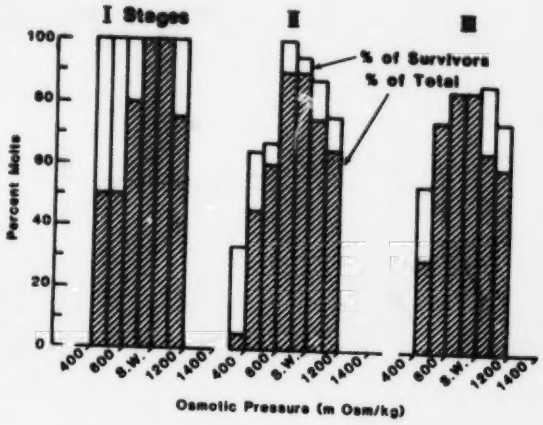

Fig. 3. Percent of molting in different salinities for 96 h. Shaded bars represent percent molts out of initial number tested (20); the sum of the shaded and unshaded bars represents percent molts out of survivors. Osmotic pressure of seawater (SW) ranged from 860-940 m Osm/kg.

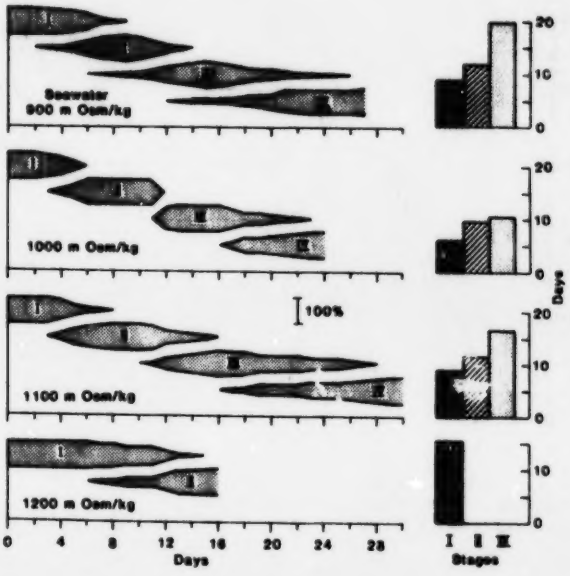

Fig. 4. Succession of the developmental stages, from stage I, in high salinities; concentrations in m Osm/kg.

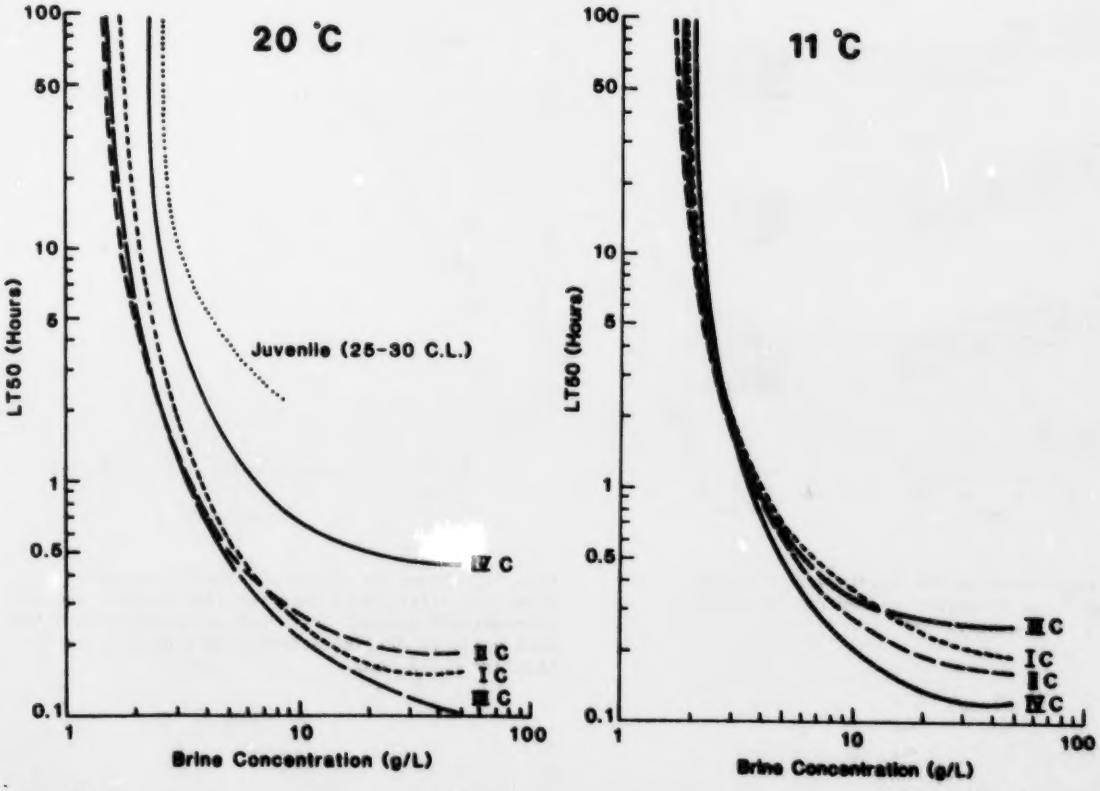

Fig. 5. Median mortality curve for larval and post larval lobsters at 20°C (a) and 11°C (b); curve fitted with Letcur program (Zitko 1982).

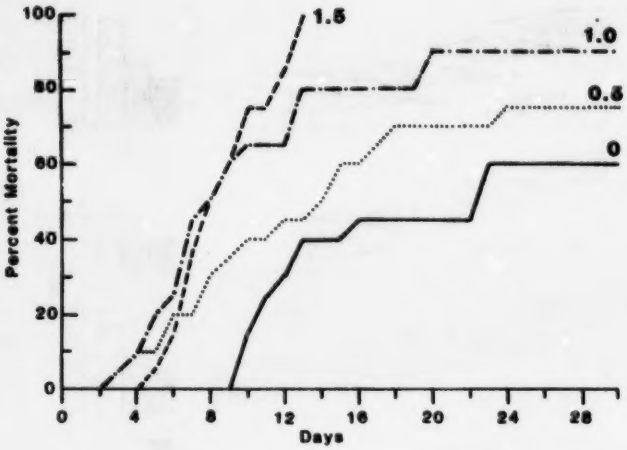

Fig. 6. Cumulative percent mortality of larvae and postlarvae, from stage I, in different concentrations (g/L) of potash ore in seawater.

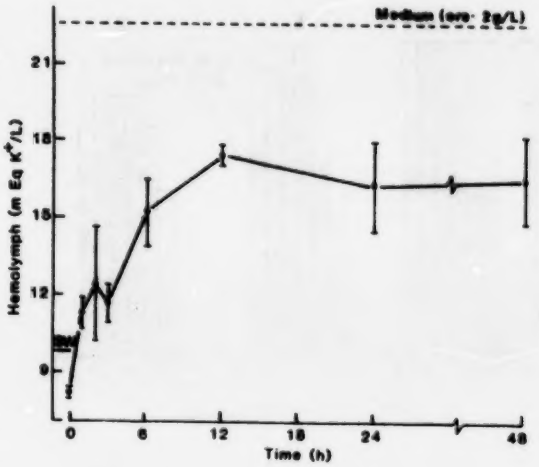

Fig. 8. Change in hemolymph K^+ concentration in juveniles after rapid transfer from SW to a medium containing 2 g ore/L SW. Each point represents the mean value of determinations from 5 animals, with standard deviations.

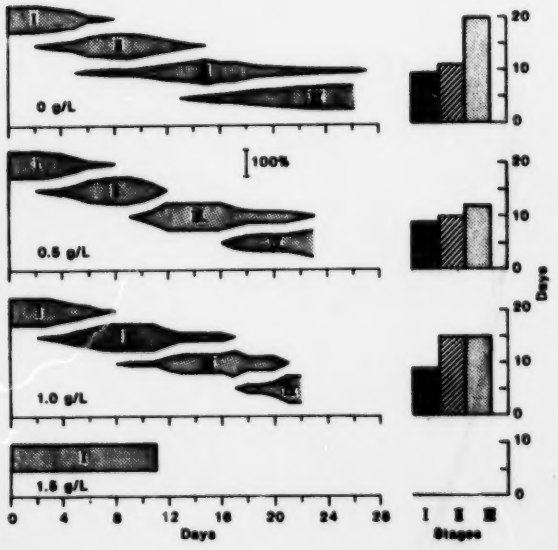

Fig. 7. Succession of the developmental stages, from stage I, in different concentrations (g/L) of potash ore in seawater.

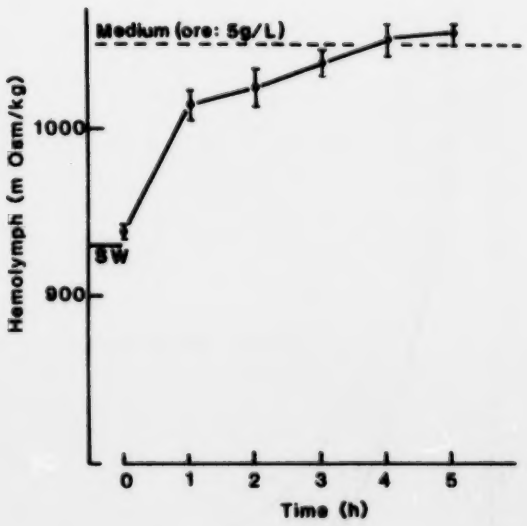

Fig. 9. Change in hemolymph osmotic pressure in juveniles after rapid transfer from SW to a medium containing 5 g ore/L SW. Each point represents the mean value of determinations from 5 animals, with standard deviations.

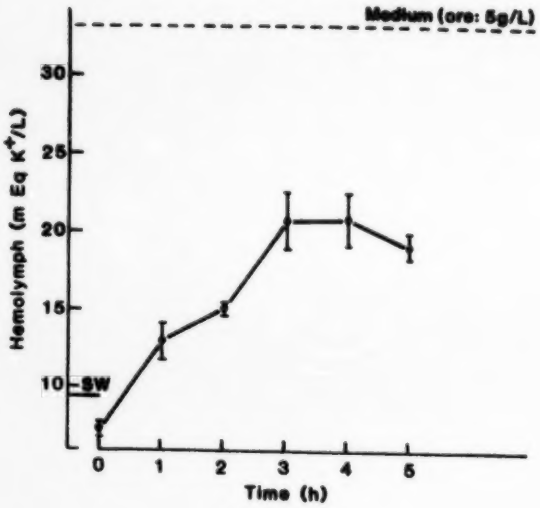

Fig. 10. Change in hemolymph K^+ concentration in juveniles after rapid transfer from SW to a medium containing 5 g ore/L SW. Each point represents the mean value of determinations from 5 animals, with standard deviations.

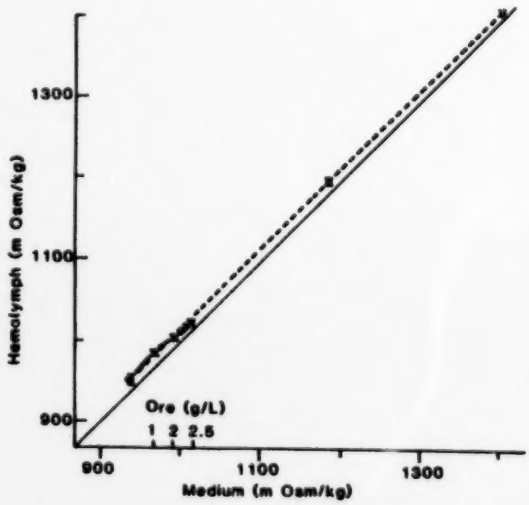

Fig. 11. Change in the osmotic pressure of the hemolymph in juveniles relative to that of the medium, after addition of Instant Ocean salts (o---) or ore (●——) to SW. The isoconcentration line is indicated. Each point represents the mean value of determinations from 7 to 10 animals, with standard deviations.

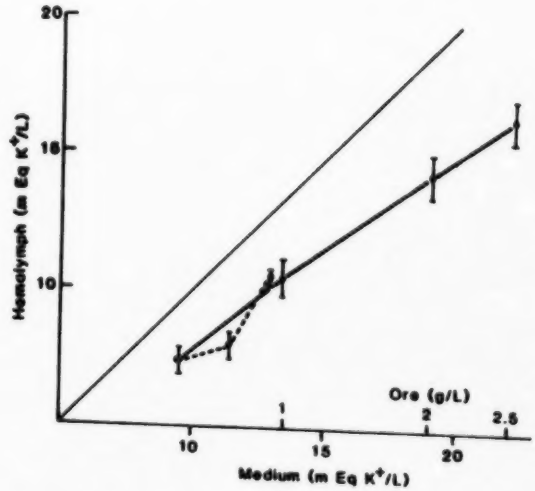

Fig. 12. Change in hemolymph K^+ concentration in juveniles relative to that of the medium, after addition of Instant Ocean salts (o---) or ore (●——). The isoconcentration line is indicated. Each point represents the mean value of determinations from 8 to 10 animals, with standard deviations.